THE EFFECTIVENESS OF IMMUNIZATION THROUGH SCARIFIED SKIN WITH THE LIVE PLAGUE VACCINE

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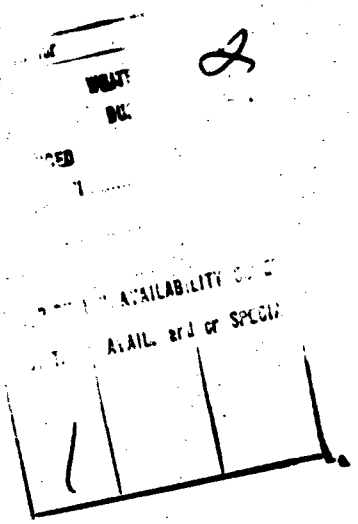

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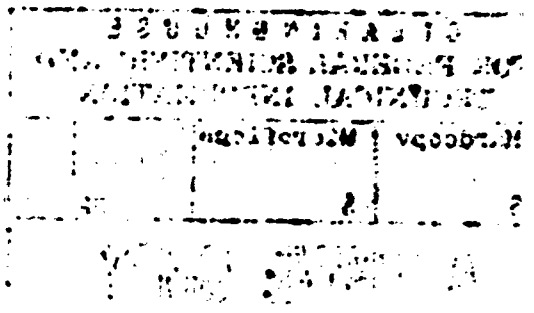

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[Following is the translation of an article by M. M. Faybich, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology Epidemiology and Immunobiology) #10, 1964, pages 125-130. The article was submitted to the editors on 6 Dec 1962. Translation performed by Sp/4 Richard M. Koplen]

Studies of immunity to plague showed that vaccination with the live vaccine through scarified skin or through the mucous lining causes the same stable nonsusceptibility as the inoculation under the skin. Also, Girard and Robic (1936) established that guinea pigs immunized with EV vaccine under the skin or through scarified skin acquired resistance and were not infected with bubonic plague and plague pneumonia, setting in during intratracheal infection. Korobkova and Kraynova (1939), in tests on intranasal EV vaccination, showed that with these methods, as also with administration of vaccine under the skin, immunization protected 90-100% of the guinea pigs from pestilential pneumonia and bubonic plague. Our investigations, which were conducted in 1941, showed that subcutaneous inoculation with dry live saccharose-gelatin vaccine of the EV strain also protected 90-100% of guinea pigs from subsequent infection through the lungs or conjunctiva, or under the skin. Korobkova (1956), on the basis of her tests, drew the conclusion that cutaneous inoculations with live EV vaccine caused an immunity in guinea pigs to the same degree (protecting over 90% of these animals) as subcutaneous inoculations with the same vaccine against infection with a virulent plague culture under the skin. In tests of Alexsandrov et al. (1958), guinea pigs immunized with plague dry aerosol vaccine from strains #1 and 17, after subcutaneous and aerial infection with the plague culture in doses of 10 and 200 Dlm, survived by 60-80%. Finally, attention is merited by the intracutaneous method of vaccination, which was suggested by Savostiniy and developed by a group of workers at the "Microbe" Institute, which proved to be sufficiently effective (citation by Korobkova).

The cited results of experimental works testify that the live vaccine, prepared from highly immunogenic strains, provides a high

specific defense against bubonic and pneumonic plague, irrespective of the method of its administration.

Our attention was drawn to the method of vaccination with live vaccine through scarified skin, which had not received, until recently, sufficient experimental foundation with various methods of infection of vaccinated animals, which are susceptible to plague.

For study we used live vaccine from a widely known EV vaccine strain (Girard and Robic, 1934). At the same time, we used live vaccines from other strains also -- #150, 148, and 149, which were obtained by us in 1945 from virulent plague cultures. The last 3 strains are different from the EV strain in that they decompose glycerine and possess more expressed immunogenic properties. Strain #150, just as the EV strain, was practically avirulent for guinea pigs; strains #148 and 149 preserved only insignificant virulence for guinea pigs (after subcutaneous administration of 20 billion microbes, the pigs died from plague in rare cases).

The live vaccine from the EV strain was used for immunization in the form of a two-day culture, incubated on the surface of a dense agar medium, or in the form of a freeze-dry preparation, which was dried in a stabilizing saccharose-gelatin medium of Faybich (1946). The live vaccine from strains #150, 149, and 148 was prepared only from the agar two-day culture. In all the tests, the suspension of vaccine cultures in physiological solution were administered to the animals one time.

For the control, some of the animals were vaccinated one time with the same preparations. Tests were conducted on white mice and guinea pigs. For vaccination through the scarified skin of white mice, a suspension of live vaccine was used, containing from 10 million up to 100 billion microbes in 1 ml. (a large part of the animals were inoculated with a suspension, containing 10-50 billion microbes in 1 ml). For vaccination of guinea pigs, a suspension containing 25 billion microbes in 1 ml. was employed. During immunization by the subcutaneous method, live vaccine was administered to mice in the quantity of 100 million and to guinea pigs 1.5 billion microbes. The vaccine was measured by the optical standard. During vaccination through the scarified skin 1-2 drops of suspension, containing the given number of microbes in 1 ml., were deposited on the surface of sheared or depilated skin in the area of the sacrum and smeared on a section with an area of 1-1.5 cm^2. After this, 12-15 scratches 1 cm. long were marked on the skin with a scarifier and with the blunt tip of the scarifier it was lightly rubbed into the skin for a half a minute. Within 30 days after inoculation, the immunized animals were infected with the virulent plague culture subcutaneously (no less than 100 Dlm) or through the lungs; in the latter case with doses which caused death, from typical primary plague

pneumonia, of all nonimmunized animals (guinea pigs and white mice) in 4-7 days after infection. Besides this, some of the pigs were infected through the conjunctiva with doses of the culture, from which all nonvaccinated animals died in 4 days. For infection we used virulent strains, 1 Dlm of which consisted of 25-50 microbes during subcutaneous infection.

In repeated tests for the comparative analysis of the effectiveness of live vaccines on a large number of white mice (not less than 350) and guinea pigs, it was established that inoculation through scarified skin caused the same immunity against pneumouic and bubonic plague as inoculation under the skin. Here, the live vaccine from the strain #150 proved to be the most effective.

White mice, which were vaccinated through the skin with live vaccines from strains EV, #150 or 149 (table 1) acquired nonsusceptibility against plague at an identical rate and no less than 90% (sometimes 100%) survived during infection with 100-500 Dlm of a virulent culture following 30 days after vaccination. Here, live vaccine from the 2-day EV culture and dry lyophilic live vaccine from the EV strain, just as vaccine from strain #150, which were deposited on the scarified skin in the form of a suspension containing 100 million microbes in 1 ml., caused the same immunity as the suspension containing 100 billion microbes in 1 ml. Thus, it is possible to draw the conclusion that only a small part of the microbes, on vaccination through the skin, are introduced into the organism of the animal, multiply in it and cause reactive changes, leading to the formation of immunity.

Tests of their infection with massive doses of a virulent culture through the lungs also testify to the high resistance of white mice, immunized through the skin. In these tests 81% (during administration of 20 billion microbes) and 83.3% (during administration of 50 billion microbes) of the vaccinated mice survived, while all the nonvaccinated mice died in 4-7 days from primary plague pneumonia.

Guinea pigs, immunized with vaccine from strain #150 or 148, both through the skin and also subcutaneously, acquired the same high degree of resistance against bubonic and pneumonic plague and 100% survived infection under the skin or through the lungs (table 2). At the same time all nonvaccinated animals, after infection through the lungs, died in 4 days, and after infection under the skin -- in 6 days. On vaccination with the 2-day EV culture and also with the dry live vaccine from the EV strain through the skin of the guinea pigs, as also in tests on white mice, identical results were obtained. All pigs which were vaccinated with this vaccine through the skin or subcutaneously proved to be resistant to further infection with large doses (no less than 100 Dlm) of a virulent culture under the skin or

through the conjunctiva. However, guinea pigs which were immunized with vaccine from the EV strain transcutaneously or subcutaneously, following their infection through the lungs in contrast to pigs inoculated with vaccines from strains #150 and 148, proved to be less resistant, while animals vaccinated through the skin were more resistant.

Studies of the reactogenicity of various live vaccines on white mice and guinea pigs showed that vaccination through the skin caused a weaker and less prolonged reaction, than subcutaneous. For the explanation of this phenomenon, it is necessary to seek the various degrees of invasiveness of vaccine cultures during their administration through and under the skin: vaccine inoculated through the skin took root at the site of administration and penetrated into the lymphatic nodes; vaccine introduced under the skin penetrated partially also into the blood and into internal organs, which could not show up in the reaction of the inoculated organism.

White mice which were immunized through the skin with vaccine from the EV strain or with vaccines from strains #150 and 149 (suspension containing from 100 million to 100 billion microbes in 1 ml) endured the indicated inoculations well, and only in certain tests, irrespective of the concentration of the suspension employed, was a small scale (no higher than 10%) death of test animals noted in the vaccinal period; the same vaccines when administered under the skin in a quantity of 100 million microbes caused considerable death of white mice: From strain EV--46.2%, from strain #150--27.8%, and from strain #149--64.3%. The results presented showed that the least reactive for white mice was the vaccine from strain #150.

In guinea pigs, vaccination through the skin caused, just as in white mice, a weaker reaction than subcutaneous: at the site of administration of vaccine, a brief local reaction developed which was accompanied by an increase of the regional nodes and an increase of temperature to 39.5° for a duration of no more than 2 days; after subcutaneous administration of 1.5 billion microbes, the temperature increased to 40° and the increase remained for a period of 5 days.

During investigation of guinea pigs which were immunized with vaccines from strain EV and #150 and killed over various times after vaccination, it was established that vaccine from strain #150, on administration through the skin, caused reactive changes principally to the same degree as the vaccine from the EV strain, but in those immunized with vaccine from strain #150, the reaction in the skin itself and in the nodes was somewhat more sharply expressed. The

fact merited attention that changes in the internal organs never occurred for the vaccines from strain EV and #150 following inoculation in regional (posteroinguinal) and iliac nodes.

As results of pathohistological investigation of the killed animals showed, which were conducted jointly by us with Chalisov, the reaction, which was detected after inoculation through the skin with vaccines from strains EV and #150 at the site of administration and also in the lymphatic nodes, emerged very quickly. Already within 6 hours after vaccination in the skin and in regional nodes, we detected the initial phenomena of serous-hemorrhagic inflammation with a predominance of the serous component, with considerable accumulation in the depth of the epidermis and skin proper of polyneuclear and histiocytic cellular elements, between which microbes were visible. Within 18 and 24 hours, the inflammatory reaction in the skin and in the regional glands was already more intensive. Within 5 days, by inspection of live guinea pigs or dissection of their dead bodies, more or less significant edema of skin with areas of hemorrhage were detected. Here, regional nodes were found to be increased up to the size of medium peas and were considerably condensated. From the surface and in a section, the nodes had a yellowish-rose color and in a number of cases dark-red. The same changes, but less intensively expressed, were detected also in the iliac nodes. Histological investigations showed that by the 5th day vesicles and miliary abscesses developed in the skin; quite large and small abscesses were found also in the regional nodes. In the cellular tissue surrounding the nodes, serous-hemorrhagic inflammatory phenomena were noted. During further histological investigations, it was revealed that in 10 days the inflammatory phenomena took on a regenerative form and led to the substitution of dead elements with young granulation tissue. Within 15 days, productive processes were noted in the skin and in the regional nodes, and within 21 days -- only certain productive reparation phenomena. Fresh scars and a normal type of node were detected during autopsy after 25 days at the site of scarification.

Besides the indicated changes in the skin, and also in the regional and iliac nodes, in the course of 15-20 hours some hyperplasia of the reticulo-endothelial cells in isolated lymphatic nodes was discovered.

Thus, the reaction to administration of the live vaccine through the skin proceeded nonmalignantly, was reversible, and comparatively brief.

Conclusions

1. A single inoculation with live plague vaccine through scarified skin imparted, to white mice and guinea pigs which were susceptible to plague, an immunity to the same degree as its inoculation under the

skin, causing during this a clinically more expressed reaction than the subcutaneous vaccination.

2. Of the live vaccines, prepared from the vaccine strain EV and strains #148, 149, and 150, the latter proved to be most effective.

3. The live vaccine prepared from strain #150 caused a reaction analogous to the reaction to the administration of the EV strain.

Literature

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Table 1

Vaccine from strain	Number of white mice survived out of the number vaccinated					
	Under the skin			Through the skin		
	Dose (in millions of microbes)	In vaccinal period	After infection under the skin with 100-500 D1m	Dose (in millions of microbes)	In vaccinal period	After infection under the skin with 100-500 D1m
EV (culture from the surface of the agar	100	42/78	4/5	100	6/9	6/6
				50	35/35	16/17
				20	40/44	28/29
				10	12/12	12/12
				1	12/12	11/12
				0.1	34/36	33/34
				0.01	12/12	11/12
EV (semiliquid culture				2	17/18	16/17
EV (dry preparation)				30	30/30	27/30
				10	22/24	20/22
				1	12/12	11/12
				0.1	12/12	11/12
No 150	100	13/18	11/13	10	11/12	10/11
				1	9/10	9/9
				0.1	12/12	12/12
No 149	100	15/42	3/4	100	9/9	8/8
				50	29/30	11/12

Legend: In the numerator -- number of surviving mice, in the denominator -- total number

Continuation of table 1.

Nonimmune white mice	Number of microbes	Number of surviving animals out of total
Control of virulence of the plague culture used for the infection of of vaccinated animals (in 6 tests)	10 100 1000 10000	15/18 4/21 0/21 0/9

Legend: Same as table 1.

Table 2

Vaccine from strain	Method of vaccination	Infection	Number of surviving g. pigs	
			Vaccinated	Nonvaccinated-Control
EV (agar culture	Under the skin Through the skin Same Same	Through the lungs Same 100 D1m under skin Through conjunctiva	7/10 15/18 5/5 10/10	0/11 0/2 0/4
EV (dry preparation	Same	100 D1m under skin	4/4	0/2
No 148	Under the skin Through the skin	Through the lungs Same	6/6 3/3	0/3
No 150	Under the skin Through the skin	Same Same	10/10 6/6	0/9 0/3

Legend: In the numerator -- number of surviving mice, in the denominator -- total number of mice in group.
Note: The animals died on the fourth day following infection through the lungs or through the conjunctiva, and on the sixth day following infection under the skin.